The Base-catalysed Rearrangement of α-Acetolactate (2-Hydroxy-2-methyl-3-oxobutanoate): a Novel Carboxylate Ion Migration in a Tertiary Ketol Rearrangement

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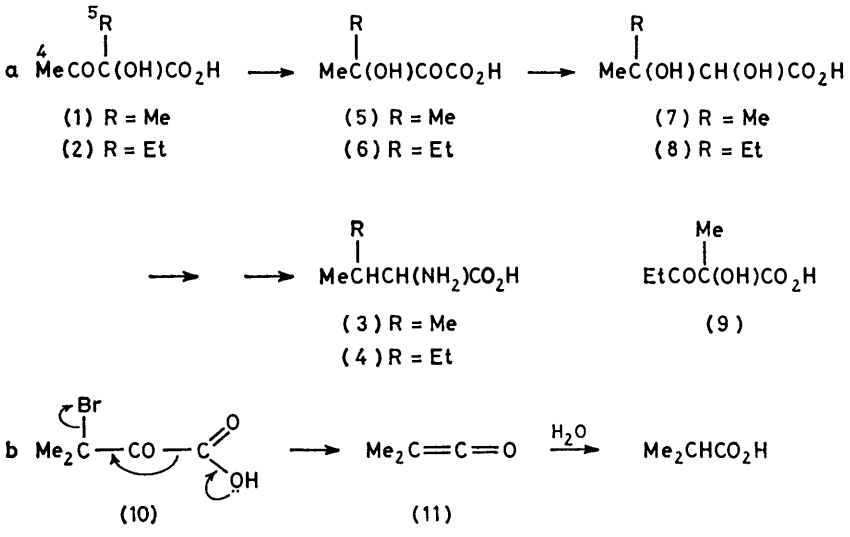
The base-catalysed racemisation of α-acetolactic acid (2-hydroxy-2-methyl-3-oxobutanoic acid) (1) has been shown to proceed by reversible tertiary ketol rearrangement with migration of the carboxylate ion rather than the methyl group. The intramolecular nature of the rearrangement was demonstrated by examination, using ^{13}C n.m.r., of the rearrangement of [1,3-$^{13}C_2$]-2-hydroxy-2-methyl-3-oxobutanoate (18).

α-ACETOLACTIC ACID (2-hydroxy-2-methyl-3-oxobutanoic acid) (1) and its homologue (2) are biological precursors of valine (3) and isoleucine (4) respectively. The conversions of the intermediates (1) and (2) into the corresponding amino-acids (3) and (4) are effected through a series of identical transformations catalysed by a common set of enzymes. The second and third steps are tertiary ketol rearrangement [(1) ⟶ (5), (2) ⟶ (6)] and reduction [(5) ⟶ (7), (6) ⟶ (8)] (Scheme 1a). Both these reactions are catalysed by a single enzyme, reductoisomerase (isomeroreductase).[1]



SCHEME 1

During an investigation of the stereoselectivity of the reductoisomerase of *Salmonella typhimurium*,[2] samples of (2*R*)-α-acetolactate (1) were prepared by hydrolysis of the corresponding optically pure methyl ester. The hydrolysate proved to be a substrate for cell-free preparations of the reductoisomerase, but the activity varied from 28 to 56% of that of the racemic material. On further examination it was found that (2*R*)-α-acetolactate (1) underwent racemisation in dilute (0.5M) sodium hydroxide solution and that the aberrant results of the enzyme assay were due to racemisation brought about by the small excess of alkali remaining after hydrolysis of the methyl ester. When methyl (2*R*)-α-acetolactate [*cf.* (1)] was hydrolysed under carefully controlled conditions in which an excess of alkali was avoided, the resulting (2*R*)-α-acetolactate was inactive in the enzyme assay.[2]

The most probable mechanism for base-catalysed racemisation of α-acetolactate (1) involved reversible tertiary ketol rearrangement [3] to the achiral 3-hydroxy-3-methyl-2-oxobutanoate [*cf.* (5)] as in the biosynthetic pathway.[1] To investigate this possibility, the hydrolysis of methyl α-acetolactate [*cf.* (1)] by an excess of sodium hydroxide in D_2O was followed by n.m.r. spectroscopy. Hydrolysis of the ester function was extremely rapid in 0.5M-alkali and was complete within 1 min at room temperature. Exchange of the 4-methyl protons was complete within 10 min. Exchange of the protons of the 5-methyl group was also observed to occur at a rate comparable with the rate of racemisation and was complete after 4 h at room temperature. This observation was clearly compatible with the proposed mechanism of racemisation (*cf.* Scheme 5a).

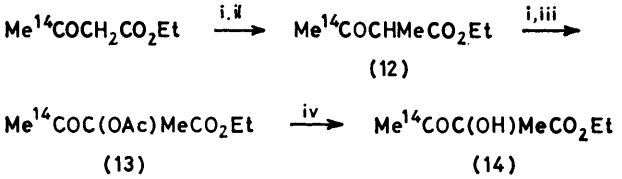
On treatment with dilute aqueous sodium hydroxide, methyl α-acetolactate underwent rapid hydrolysis to methanol and α-acetolactate anion [as (1)]. The latter was relatively stable but underwent slow decomposition to acetoin.

Hydrolysis of methyl α-acetolactate [*cf.* (1)] was

accompanied by β-ketoester cleavage to lactic and acetic acids. The fraction of the initial ester undergoing this competitive cleavage during hydrolysis varied from *ca.* 10% in 0.5M-sodium hydroxide solution to 50% in 2.5M-sodium hydroxide solution.

The reversible rearrangement of α-acetolactate (1) is degenerate in that the reactant and product have identical constitutions. However, rearrangement of the isoleucine precursor (2) was expected to give the isomer (9). Accordingly the rearrangement of 2-ethyl-2-hydroxy-3-oxobutanoate (2) in aqueous alkali was followed by n.m.r. spectroscopy. The appearance of a new quartet–multiplet combination attributable to the ethyl group in the rearrangement product (9), and of a new singlet attributable to the *C*-methyl group, confirmed that the rearrangement (2) ⟶ (9) had occurred.

The foregoing evidence clearly indicated that racemisation of α-acetolactate (1) was accompanied by, and therefore presumably due to, tertiary ketol rearrangement. However there was as yet no evidence for the participation of 3-hydroxy-3-methyl-2-oxobutanoate (5) in this rearrangement, since a signal attributable to the

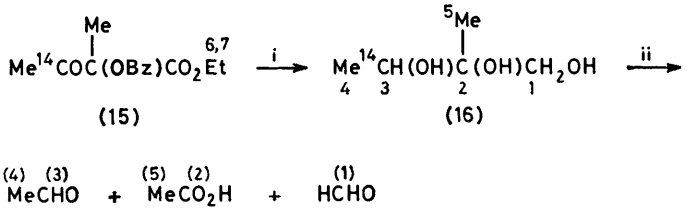


SCHEME 2 Reagents: i, NaOEt; ii, MeI; iii $(AcO)_2$; iv, $NaHCO_3$–H_2O

gem-dimethyl system of (5) had not been observed during the n.m.r. study. If this species were involved its equilibrium concentration must have been very low, a consideration that led to the prediction that treatment of 3-hydroxy-3-methyl-2-oxobutanoate (5), prepared by an independent route, with base should result in rapid and quantitative conversion into α-acetolactate (1). The required compound (5) was obtained by treatment of the bromo-acid (10) with alkali following an established procedure.[4] The acid (10) proved to be unstable in water. The n.m.r. spectrum of an aqueous solution consisted initially of a singlet attributable to the *gem*-dimethyl system. However, this signal slowly diminished in intensity in step with the appearance of a doublet–septet system, the signals of which were entirely coincident with those of added isobutyric acid. This transformation is most readily explained as proceeding *via* the intermediate dimethylketen (11) (Scheme 1b). At the same time a singlet attributable to the hydrolysis product (5) appeared. The final ratio of isobutyric acid to hydrolysis product (5) was *ca.* 2 : 1.

The acid (5) proved to be stable in 0.5M-alkali; its n.m.r. spectrum remained unchanged during 28 h. Accordingly 3-hydroxy-3-methyl-2-oxobutanoate (5) could not be invoked as an intermediate in the rearrangement of α-acetolactate (1).

An alternative explanation was that rearrangement took place by carboxylate ion migration rather than by methyl migration. Since the rearrangement of α-acetolactate (1) is degenerate, this could only be proved by labelling studies. Accordingly ethyl [3-^{14}C]-α-acetolactate (14) was synthesised from ethyl [3-^{14}C]-3-oxobutanoate as shown in Scheme 2. α-Acetolactate (1)

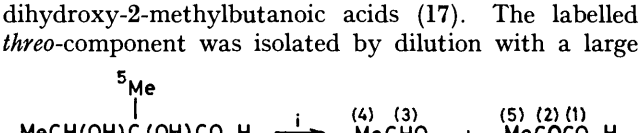


SCHEME 3 Reagents: i, $LiAlH_4$; ii, $NaIO_4$

for reductiosomerase assays is usually prepared by the method of Krampitz [5] in which an acetoacetic ester is acetoxylated with lead tetra-acetate. However, we found this method to be unpredictable on a small scale and therefore not suitable for a radiotracer synthesis. Accordingly the alternative procedure (Scheme 2) was developed in which the anion of the acetoacetic acid ester was acetoxylated with acetyl peroxide. Subsequently benzoyl peroxide was found to give reproducibly better yields and was used in later preparations of α-acetolactate derivatives.

To confirm that the ^{14}C label was located exclusively at C-3 in the labelled ethyl α-acetolactate (14), and that it had not become distributed between C-1 and -3 by reverse Claisen ester condensation under the conditions of the α-methylation step, a sample of the ethyl [3-^{14}C]-2-methyl-3-oxobutanoate (12) was benzoyloxylated by treatment of the sodio-derivative with benzoyl peroxide, and the product (15) was rigorously purified by high-pressure liquid chromatography (h.p.l.c.). The ester (15) was reduced with lithium aluminium hydride to the triol (16) which was oxidised with sodium periodate to acetaldehyde (C-3,-4), formaldehyde (C-1), and acetic acid (C-2,-5) (Scheme 3). Only the acetaldehyde was labelled, proving that the ^{14}C-label in the α-acetolactate was confined exclusively to the C-3,-4 component.

Ethyl [3-^{14}C]-α-acetolactate (14) was hydrolysed with an excess of alkali and kept in the presence of *ca.* 0.5M-alkali at room temperature for 5 h. The product was reduced with sodium borohydride to stabilise the α-acetolactate as a mixture of *erythro*- and *threo*-2,3-dihydroxy-2-methylbutanoic acids (17). The labelled *threo*-component was isolated by dilution with a large



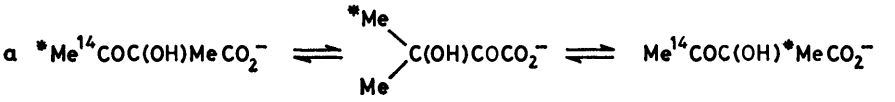
SCHEME 4 Reagent: i, $NaIO_4$

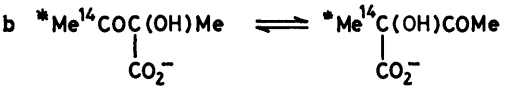
excess of the dicyclohexylamine salt of *threo*-2,3-dihydroxy-2-methylbutanoic acid [as (17)] and recrystallisation to constant activity. The radiochemically pure dihydroxy-acid was oxidised with periodate to acetaldehyde and pyruvic acid (Scheme 4) which contained

respectively 71 and 34% of the activity of the dihydroxy-acid. If the methyl migration mechanism were operative the label would have been entirely confined to the acetaldehyde fragment (*cf.* Scheme 5a) whereas distribution of activity between both fragments from the periodate oxidation confirmed the occurrence of the carboxylate ion migration (Scheme 5b).

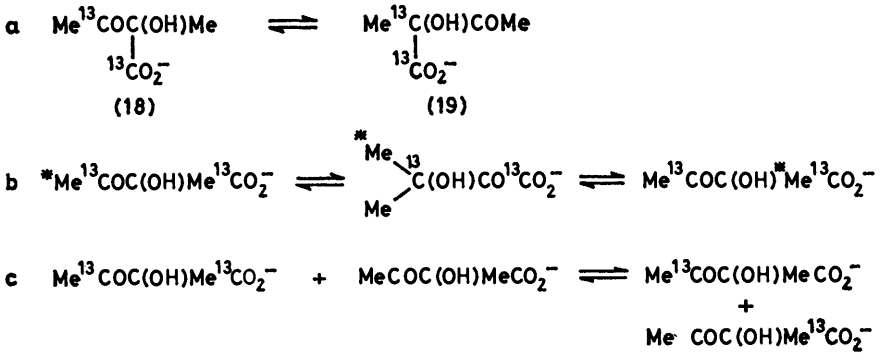
To obtain further confirmation of the carboxylate ion migration and to obtain evidence on the intra- or inter-molecularity of the rearrangement, a study using ^{13}C-labelled α-acetolactate (1) was undertaken. The objective was the synthesis and rearrangement of [1,3-$^{13}C_2$]-α-acetolactate (18) (Scheme 6). In the presence of an excess of unlabelled α-acetolactate (1), the intramolecular migration of the carboxylate ion would lead to a 1 : 1 mixture of [1,3-$^{13}C_2$]-(18) and [1,2-$^{13}C_2$]-α-acetolactate (19) (Scheme 6a). Formation of the latter species would readily be confirmed by the coupling in the ^{13}C n.m.r. spectrum between C-1 and -2. Rearrangement with methyl migration would give a product with the same labelling pattern as the starting material (Scheme 6b). Rearrangement with intermolecular transfer of the carboxylate ion would give a mixture of singly labelled species (Scheme 6c).

To maximise the efficiency with which the labelled starting material was elaborated to α-acetolactate (1), several modifications of the existing syntheses were introduced (Scheme 7). A major feature was the protection of the carboxy-group as the benzyl ester, which not only decreased the volatility of low molecular weight intermediates, but also served as a u.v.-sensitive label in the purification of intermediates by h.p.l.c. In trial experiments, acetic acid was converted into benzyl acetate by treatment with an excess of benzyl bromide in methanol–*NN*-dimethylacetamide in the presence of tetramethylammonium hydroxide.[6] The product, consisting of benzyl acetate, benzyl bromide, and benzyl methyl ether, was purified by chromatography on silica gel to give pure benzyl acetate in 75% yield. Claisen ester condensation of benzyl acetate with lithium hydride as base[7] gave benzyl 3-oxobutanoate in 56% yield after purification by h.p.l.c. This was converted by methylation and benzoylation with benzoyl peroxide into the ester [*cf.* (21)] in 71% yield. The synthesis of the

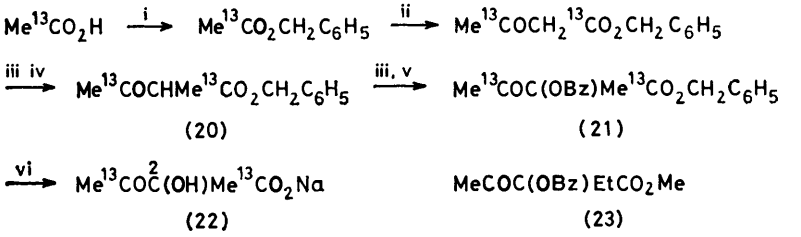




SCHEME 5



SCHEME 6



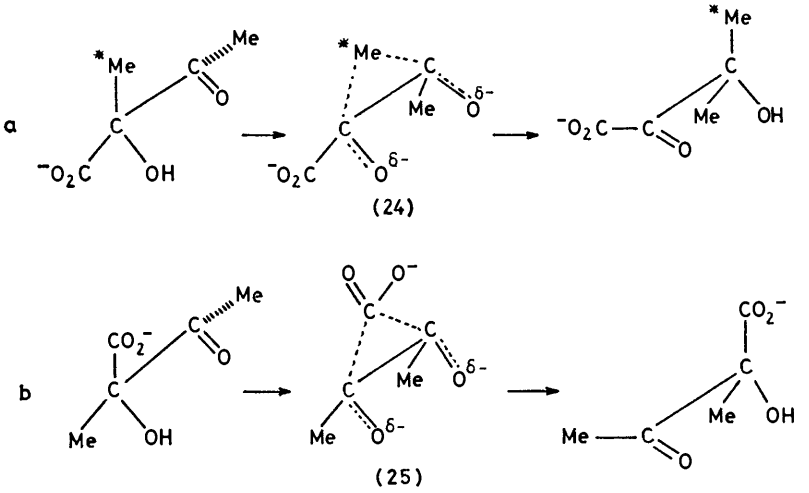
SCHEME 7 Reagents: i, $C_6H_5CH_2Br$–$Me_4N^+OH^-$; ii, LiH; iii, Na; iv, MeI; v, $(BzO)_2$; vi, NaOH–H_2O

doubly labelled ester (21) was carried out by a similar procedure. From the initial esterification, benzyl [1-^{13}C]acetate was obtained in admixture with benzyl methyl ether. The mixture was used in the subsequent Claisen ester condensation without further purification. The product, benzyl [1,3-$^{13}C_2$]-3-oxobutanoate, was diluted seven-fold with unlabelled material and the mixture was converted as described above into the ester (20) and thence into benzyl [1,3-$^{13}C_2$]-2-benzoyloxy-2-methyl-3-oxobutanoate (21).

The doubly labelled ester (21) was hydrolysed with two equivalents of sodium hydroxide solution to give a mixture of sodium [1,3-$^{13}C_2$]-α-acetolactate (22), benzyl alcohol, and sodium benzoate. The ^{13}C n.m.r. spectrum of the resulting solution included singlets at δ 213.2 and 177 due to the Me^{13}CO and $^{13}CO_2^-$ groups respectively, and a weak natural abundance singlet at δ 83.4 due to C-2 [*cf.* (22)]. The neutral solution was brought to 0.2 mol dm^{-3} in sodium hydroxide by the addition of a dilute aqueous solution. After 90 min the n.m.r. spectrum indicated that equilibrium had been reached. (Attainment of equilibrium was faster than in the corresponding ^{1}H n.m.r. experiments owing to the higher temperature at which the ^{13}C n.m.r. experiment was conducted.) At this time the signal due to the C-3 carbonyl carbon had diminished in intensity, the signal due to the carboxylate carbon, C-1, had changed to a singlet flanked by a doublet (J 50.4 Hz) of approximately equal intensity, and the natural abundance signal due to C-2 was flanked by a relatively intense doublet (J 50.4 Hz). The ^{13}C n.m.r. spectrum was therefore clearly consistent with the production of a mixture of [1,3-$^{13}C_2$]- and [1,2-$^{13}C_2$]-α-acetolactate, confirming first that migration of the carboxylate group had taken place and secondly that the rearrangement was intramolecular (*cf.* Scheme 6).

There is thus a marked contrast between the tertiary ketol rearrangement with methyl migration of the biosynthetic pathway [(1) ⟶ (5)] and the *in vitro* rearrangement with carboxylate ion migration (Scheme 5b). This suggests that if the mechanisms of the two transformations are broadly similar, the enzyme takes advantage of the stereoelectronic requirements of the rearrangement to force the substrate to adopt the conformation at the active site that favours methyl rather than carboxylate migration.

The configuration of the product of the rearrangement, 3-hydroxy-3-methyl-2-oxobutanoate (5) in the *in vivo* pathway is not known. However, it can be inferred from the configuration (2*R*,3*R*) [8] of the corresponding intermediate (6) of the isoleucine pathway, in which the migrating ethyl group occupies the position corresponding to the *pro-R* methyl group of the valine precursor (5). The stereochemistry of the transition state of the rearrangement *in vivo* * can thus be depicted as in (24) (Scheme 8a). A feature of this structure is the eclipsed



SCHEME 8

arrangement of the two C–O bonds. Since the base-catalysed rearrangement of α-acetolactate (1) *in vitro* results in racemisation, the corresponding transition state must be partly, if not wholly, represented by the *meso*-structure (25) (Scheme 8b) in which the same eclipsed arrangement of the C–O bonds is found. Racemisation of (2*R*)-α-acetolactate (1) in alkaline solution was found to be first order in (*R*)-α-acetolactate. Racemisation might be stereospecific, with the *R*-isomer giving exclusively *S*-isomer and *vice versa*. Alternatively, racemisation might be non-stereospecific, *R*-isomer giving rise to *R*- and *S*-isomers in a ratio determined by the relative magnitudes of the rate constants k_1 and k_2 for conversion of *R*-isomer into *S*- and *R*-isomer respectively (Scheme 9). However, it is

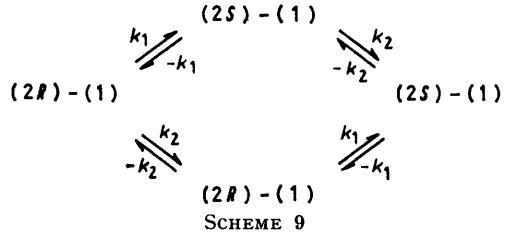
$$\ln \alpha = -2k_1t + \ln \alpha_0 \qquad (1)$$

readily shown that the rate of racemisation is independent of k_2 and is given by equation (1) where α_0 is the

* The precise nature of the species undergoing rearrangement *in vivo*, is not known. An anionic species, as in the *in vitro* rearrangement, is assumed only to illustrate the stereochemistry of the *in vivo* rearrangement.

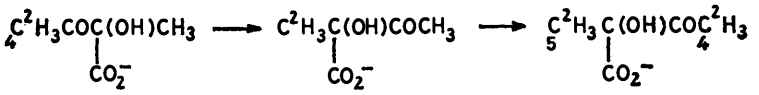
initial optical rotation and α is the optical rotation after time t.

In the base-catalysed deuterium exchange described above, the rate of exchange of the protons of the acetyl methyl group of α-acetolactate (1) was much greater than the rate of rearrangement. Consequently the reaction observed by n.m.r. was exclusively the conversion



SCHEME 9

of [4-$^2H_3$]-α-acetolactate into [5-$^2H_3$]-α-acetolactate (Scheme 10), since the reverse reaction was not observable owing to the rapid conversion of [5-$^3H_2$]-α-acetolactate, once formed, into [4,5-$^2H_6$]-α-acetolactate (Scheme 10). The reaction observed in the n.m.r.



SCHEME 10

experiment, ignoring possible isotope effects, was therefore that given in Scheme 11. The corresponding

SCHEME 11

rate expression is given in equation (2) where a_0 = initial concentration of [4-$^2H_3$]-α-acetolactate and a = con-

$$\ln a = -(k_1 + k_2)t + \ln a_0 \qquad (2)$$

centration after time t. Thus the stereospecificity of the rearrangement could, in principle, be determined by comparing the specific rates of racemisation and deuterium exchange, if suitable corrections could be made for isotope effects. For a completely stereospecific rearrangement, with (2*R*)-α-acetolactate giving rise exclusively to the 2*S*-isomer and *vice versa* (k_2 0, Scheme 9), the specific rate of racemisation would be twice that of deuterium exchange [*cf.* equations (1) and (2)], whereas for a completely non-stereospecific rearrangement ($k_1 = k_2$ in Scheme 9), the two rates would be equal. If the 2*R*-isomer were preferentially converted into the 2*R*-isomer ($k_2 > k_1$, Scheme 9), the rate of racemisation would be slower than the rate of exchange. However, since the rates of racemisation and deuterium exchange cannot reliably be compared, the stereospecificity of the *in vitro* rearrangement is under investigation by a method which does not rely on kinetic data. The stereochemistry of the *in vivo* rearrangement with respect to the configurational position adopted by the migrating methyl groups is also under investigation.

While these studies were in progress, it was reported that in the benzilic acid rearrangement of the 2,3-dioxobutanoate ion, migration of the carboxylate group takes precedence over methyl migration.[9] It thus appears that in 1,2-anionic rearrangements, the migratory aptitude of the carboxylate ion exceeds that of the methyl group.

The base-catalysed reacemisation of α-acetolactate (1) was discovered independently by Hill and his coworkers.[10] We are grateful to Professor Hill for a generous exchange of information.

EXPERIMENTAL

All m.p.s are corrected. I.r. spectra were determined with Hilger H900 Infrascan and Perkin-Elmer 357 grating spectrometers, and n.m.r. spectra with a JEOL MH-100 (^1H), and pulsed Fourier-transform spectra (^{13}C) with Varian XL-100 and JEOL PFT-100 spectrometers for solutions in deuteriochloroform with tetramethylsilane as internal standard or in water or D_2O with sodium 3-trimethylsilylpropane sulphonate as internal standard. Analytical g.l.c. was performed using a Pye 104 gas–liquid chromatograph. H.p.l.c. separations were carried out with a Waters Associates Liquid Chromatograph M6000 solvent delivery system. All radiochemicals were purchased from the Radiochemical Centre, Amersham, and ^{13}C-labelled compounds from Prochem Ltd. Radioactivity determinations were made with a Packard Tri-Carb series 2000 spectrometer.

Methyl 2-Benzoyloxy-2-ethyl-3-oxobutanoate (23).—A solution of methyl 2-ethyl-3-oxobutanoate (4.0 g) in dry ether (20 cm^3) was stirred vigorously with sodium wire (0.65 g) until all the sodium had reacted. The mixture was cooled to 0 °C and was treated dropwise with a solution of benzoyl peroxide (6.8 g) in benzene (50 cm^3) over 20 min. The mixture was stirred for 2 h at 0 °C, allowed to warm to room temperature overnight, washed with water (4 × 50 cm^3), dried ($MgSO_4$), and evaporated to give a mixture which consisted of methyl 2-benzoyloxy-2-ethyl-3-oxobutanoate (23) (6.9 g) together with unchanged benzoyl peroxide. The product was purified by either (a) preparative t.l.c. or (b) h.p.l.c. (a) The crude product (200 mg) was purified by chromatography on two 20 × 20 cm plates each coated with Kieselgel PF_{254} (20 g). The band corresponding to the ester was eluted with chloroform and the product was purified by bulb-tube distillation (145 °C, 0.1 mmHg) to give the *ester* (23) (104 mg. 54% overall) (Found: C, 63.65; H, 6.15. $C_{14}H_{16}O_5$ requires C, 63.6; H, 6.1%), $\nu_{max.}$ (neat) 1 755 (CO·OEt), 1 735 (sh, ArCO), and 1 720 (MeCO) cm^{-1}; δ_H 0.94 (3 H, t, *J* 6.5 Hz, *Me*CH_2), 2.27 (2 H, q, *J* 6.5 Hz, MeCH_2), 3.66 (3 H, s, MeO), and 7.2—8.0 (5 H, m, C_6H_5); δ_C 7.9 (C-6), 27.1 (C-4), 27.4 (C-5), 53.0 (C-8), 88.6 (C-2), 128.5, 129.0, 129.9, 133.7, (C-2′—7′), 165.0, 167.9 (C-1, C-1′), and 200.7 p.p.m. (C-3). (b) The product was purified by h.p.l.c. on a 4-ft × $\frac{3}{8}$-in column of Bondapak

C_{18} Porasil B with methanol–water (2 : 1) as eluant. The eluate was concentrated to remove methanol and the aqueous residue was extracted three times with an equal volume of chloroform. The extracts were dried ($MgSO_4$) and evaporated to give the ester (15) (50%; purity >95%, n.m.r.).

Rearrangement of 2-Ethyl-2-hydroxy-3-oxobutanoic Acid (2).—The ester (23) (100 mg) was dissolved in methanol (1.7 cm³) and treated dropwise with stirring with sodium hydroxide solution (0.82M. 2.4 cm³). The solution was stirred for 30 min, the methanol was removed *in vacuo*, and the solution (0.5M in NaOH) was transferred to an n.m.r. tube.

^{1}H N.m.r. spectra were determined at intervals during 7 h. The triplet (δ *ca.* 0.9) attributable to the $MeCH_2$ group gradually diminished in intensity and a downfield triplet (δ *ca.* 1.0) attributable to $MeCH_2CO$ gradually grew to equal it in intensity after 2 h. This change was accompanied by the emergence of a multiplet (presumed quartet) near δ 2, attributable to $MeCH_2CO$.

Racemisation of (2R)-α-*Acetolactate* (1) *in Alkaline Solution*.—Methyl (2*R*)-α-acetolactate (2.049 mg) was dissolved in sodium hydroxide solution (0.5M, 3 cm³). The c.d. of the solution was determined at intervals during 5 h at 21 °C. Initially the solution showed maxima at 269 (Δε + 0.23) and 304 nm (Δε − 0.31). Racemisation was followed by observing the change with time of the maximum at 304 nm. The rate of racemisation was approximately first order in acetolactate with a half-life of 70 min. A duplicate experiment using 2.181 mg of the ester gave an identical result. The c.d. measurements were kindly made by Dr. P. M. Scopes and Professor W. Klyne.

Hydrolysis of Methyl α-*Acetolactate in Sodium Hydroxide Solution*.—In solution in H_2O, methyl α-acetolactate [*cf.* (1)] gave the following n.m.r. spectrum; δ 3.83 (s, MeO), 2.37 (s, MeCO), and 1.65 (s, *Me*COH). Methyl α-acetolactate (18.5 mg) in sodium hydroxide solution (0.5M, 0.4 cm³) gave, after 1 min, the following n.m.r. spectrum; δ 3.39 (3 H, s, *Me*OH), 2.29 (*ca.* 3 H, s, MeCO), and 1.50 (*ca.* 3 H, s, *Me*COH). In addition, peaks attributable to $MeCO_2H$ [*ca.* 10%, δ 1.95 (s)] and *Me*CH(OH)CO_2H [*ca.* 10%, δ 1.37 (d, *J* 7 Hz)] were observed. After 10 days, in addition to the signal due to *Me*OH, the principal additional signals were attributable to acetoin, δ 2.26 (s) and 1.41 (d, *J* 7 Hz). When methyl α-acetolactate (14.3 mg) was dissolved in sodium hydroxide solution (2.5M, 0.4 cm³) a similar n.m.r. spectrum was obtained except that the signals attributable to acetic and lactic acids corresponded to 50% β-ketoester cleavage. The signals at δ 1.95 and 1.37 were exactly coincident with those due to added acetic and lactic acids.

Hydrolysis of Methyl α-*Acetolactate in* NaOD–D_2O.—Methyl α-acetolactate (19.6 mg) was dissolved in a solution of sodium hydroxide in D_2O (0.5M, 0.55 cm³). After 5 min, signals due to MeOD (δ 3.28), CH_3CO, CH_2DCO, CHD_2CO [δ 2.20br (s) and 1.41 (s, MeCOD)] were observed. The signal at δ 2.20 on integration was found to have 17% of the magnitude of the MeOD signal, corresponding to 84% H–D exchange. In addition, signals at δ 1.88 (s, $MeCO_2D$) and 1.28 [s, MeCD(OD)CO_2D] were observed which corresponded to 16% β-ketoester cleavage. After 15 min the signal at δ 2.20 due to the MeCO protons was no longer observable. During 2 h, the signal δ 1.41 due to MeCOD gradually diminished in intensity. After 2 h, this signal had decreased to 18% of the magnitude of the signal due to MeOD, and after 16 h it was no longer observable.

Alkaline Hydrolysis of 3-*Bromo*-3-*methyl*-2-*oxobutanoic Acid* (10).—The bromo-acid (10) was prepared according to the published procedure,[4] δ($CDCl_3$) 2.08 (6 H, s, Me_2), 9.91br (1 H, s, CO_2H), δ(H_2O) 1.86 ($Me_2CBrCOCO_2H$) changing during 5 h at room temperature to 1.15 (d, *J* 7 Hz, Me_2CHCO_2H), 2.62 (septet, *J* 7 Hz, Me_2CHCO_2H), and 1.44 [s, $Me_2C(OH)COCO_2H$]. The signals at δ 1.15 and 2.62 were entirely coincident with those of added isobutyric acid. The acid (10) in sodium hydroxide solution (5% w/v, 0.4 cm³) gave a singlet at δ 1.39. The spectrum remained unchanged over 28 h.

Synthesis and Rearrangement of [3-^{14}C]-α-*Acetolactate* [cf. (1)].—A solution of ethyl [3-^{14}C]-3-oxobutanoate (350 mg; 12 μCi) in dry ether (5 cm³) was treated with a solution of sodium ethoxide in ethanol (1M, 2 cm³) and iodomethane (400 μl). The mixture was boiled under reflux for 4 h, distilled to remove most of the ether, and treated with benzene (10 cm³). The solution was washed with water (1 × 5 cm³; 1 × 2.5 cm³) and the washings were re-extracted with benzene (1 × 5 cm³). The combined benzene solutions were dried ($MgSO_4$) and filtered. The drying agent was washed with ether (5 cm³) and the washings were added to the benzene solution. To the cooled (0 °C), stirred solution, a solution of sodium ethoxide in ethanol (1M, 2 cm³) was added, followed by an ethereal solution of acetyl peroxide [prepared from acetic anhydride (1.83 g) and barium peroxide (1.8 g) in dry ether (30 cm³)]. The mixture was stirred at 0 °C for 1 h and then was allowed to warm to room temperature with stirring over 3.5 h. The solution was washed with water (2 × 5 cm³), the aqueous washings were re-extracted with ether (10 cm³), and the combined ethereal extracts were dried (Na_2SO_4) and evaporated to give crude ethyl [3-^{14}C]-2-acetoxy-2-methyl-3-oxobutanoate (13) as an oil. This was treated with sodium hydrogencarbonate solution (0.2M, 30 cm³) and the mixture stirred for 2 h to hydrolyse the acetoxy-function. The solution was extracted with ethyl acetate (6 × 10 cm³), and the extracts were dried (Na_2SO_4) and evaporated to give crude ethyl [3-^{14}C]-2-hydroxy-2-methyl-3-oxobutanoate (14) as an oil (180 mg). A portion of this material was purified by preparative t.l.c. [Kieselgel PF_{254}; solvent system ether–light petroleum (b.p. 40—60 °C) (2 : 3)]. The identity of the isolated material was confirmed by n.m.r. The ester (14 mg, *ca.* 1.2 μCi) was treated with sodium hydroxide solution (3%, 0.4 cm³) for 5 h. Ethanol (5 cm³) was added, followed by sodium borohydride (30 mg). The mixture was stirred for 1 h, treated with sodium borohydride (30 mg) and stirred for a further 30 min. The ethanol was removed under reduced pressure and the residual aqueous solution was acidified (Congo Red) with hydrochloric acid (2M), treated with the dicyclohexylamine salt * of *threo*-2,3-dihydroxy-2-methylbutanoic acid (10 mg) and extracted continuously with ether for 24 h. The residue was treated with the dicyclohexylamine salt of *threo*-2,3-dihydroxy-2-methylbutanoic acid [11] (2.02 g) and the salt was recrystallised (ethanol–acetone) to constant activity (9 860 d.p.m. mmol^{-1}). To a solution of this salt (200 mg) in water (8 cm³) was added sodium periodate (170 mg). After 20 min, water (12 cm³) was added and 5 cm³ of the solution was distilled into a solution of dimedone (400 mg) in water (100 cm³). The mixture was set aside overnight and filtered to give the dimedone derivative of acetaldehyde (130 mg), m.p. 141—142 °C, which was recrystallised (ethanol–water)

* M.p. 175—177 °C (Found: C, 64.6; H, 10.5; N, 4.5. Calc. for $C_{17}H_{33}NO_4$: C, 64.7; H, 10.5; N, 4.45%).

to constant activity [7 040 d.p.m. mmol^{-1}; 71.3% of the activity of the dihydroxy-acid (17)]. The remaining solution was evaporated to dryness and the residue dried over silica gel under reduced pressure. The residue was extracted with boiling acetone (2 × 10 cm^3). The extracts were filtered and concentrated to give the dicyclohexylamine salt of pyruvic acid, plates, m.p. 163—164 °C (decomp.), identical with authentic material (m.p. and mixed m.p.) (Found: C, 66.9; H, 10.1; N, 5.15. Calc. for $C_{15}H_{27}NO_3$: C, 66.9; H, 10.1; N, 5.2%). The salt was recrystallised (acetone) to constant activity [3 340 d.p.m. mmol^{-1}; 33.8% of the activity of the starting dihydroxy-acid (17)].

Synthesis and Degradation of Ethyl [3-^{14}C]-2-*Methyl*-3-*oxobutanoate*.—Ethyl [3-^{14}C]-2-methyl-3-oxobutanoate, prepared as described above, was converted into ethyl [3-^{14}C]-2-benzoyloxy-2-methyl-3-oxobutanoate and purified by h.p.l.c. as described above for the preparation of methyl 2-benzoyloxy-2-ethyl-3-oxobutanoate (23). The labelled ester (325 mg; 45 700 d.p.m. mmol^{-1}) in dry ether (40 cm^3) was boiled under reflux with lithium aluminium hydride (1.0 g) for 20 h. The excess of reagent was destroyed by the addition of water, the resulting slurry was filtered, the ether was removed, and the aqueous residue was applied to a column of Dowex 50W-X8 cation exchange resin (H^+). The eluate was collected until it no longer gave a reaction with starch–periodate reagent. The eluate (100 cm^3) was treated with sodium periodate (324 mg), followed, after 40 min, by sodium arsenite (160 mg). The solution was made just alkaline (phenolphthalein) by the addition of dilute sodium hydroxide solution and distilled until 60 cm^3 of distillate had been collected. This was added to a solution of dimedone (800 mg) in water (200 cm^3) and left to stand overnight. The mixture of derivatives was filtered off and washed with water. The dried mixture (285 mg) was heated on a steam-bath in acetic acid (10 cm^3) for 6 h. The product was poured into water (200 cm^3), the mixture was allowed to stand overnight, the derivatives were filtered off and washed with sodium hydroxide solution (2M; 25 cm^3) and water. The alkaline washings were brought to pH 7 by the addition of hydrochloric acid (2M) and the precipitate was filtered off and recrystallised (ethanol–water) to give the dimedone derivative of formaldehyde (130 mg), m.p. 189—191.5 °C.

The alkali-insoluble component was crystallised (ethanol–water) to give the anhydrodimedone derivative of acetaldehyde (65 mg), m.p. 175—176.5 °C. The residual solution from the distillation of the aldehydes was acidified (Congo Red), filtered, and extracted continuously with ether containing a little mercury for 40 h. The ethereal extract was decanted from the mercury, treated with water (5 cm^3), and titrated, with stirring, against barium hydroxide solution (phenolphthalein). The ether was removed, the residual aqueous solution was treated with solid carbon dioxide, filtered, concentrated to 1 cm^3, and treated with ethanol. The amorphous precipitate was recrystallised from ethanol–water to give barium acetate monohydrate (needles). All three derivatives were recrystallised to constant activity: barium acetate monohydrate, 538 d.p.m. mmol^{-1} (0.6%); formaldehyde dimedone derivative, 46 d.p.m. mmol^{-1} (0.08%); anhydrodimedone derivative of acetaldehyde, 45 400 d.p.m. mmol^{-1} (99.4%).

Synthesis of Benzyl 2-*Benzoyloxy*-2-*methyl*-3-*oxobutanoate* [cf. (21)].—(a) *Benzyl* 2-*methyl*-3-*oxobutanoate* [cf. (20)]. Sodium wire (1.2 g) was stirred with benzyl alcohol (30 cm^3) and dry ether (15 cm^3) until it had all reacted. Iodomethane (20 g) and benzyl 3-oxobutanoate (10 g) were added and the mixture was boiled under reflux for 2 h. The excess of iodomethane, together with some ether, were evaporated, ether (200 cm^3) was added, and the solution filtered. The filtrate was washed with water (2 × 50 cm^3), dried ($MgSO_4$), and evaporated. The residual oil (20.5 g) was twice distilled to give benzyl 2-methyl-3-oxobutanoate [*cf.* (20)], b.p. 84—86 °C at 0.01 mmHg (lit.,[12] 145—148 °C at 9 mmHg). It was found difficult to free the ester from a small amount of impurity; accordingly for analysis the 2,4-dinitrophenylhydrazone was prepared (see below), δ 1.34 (3 H, d, *J* 7.2 Hz, *Me*CH), 2.13 (3 H, s, MeCO), 3.52 (1 H, q, *J* 7.2 Hz, CH), 5.16 (2 H, s, CH_2), and 7.36 (5 H, s, C_6H_5). The 2,4-dinitrophenylhydrazone, after purification by chromatography on neutral alumina in toluene, crystallised (ethanol) as yellow-orange *needles*, m.p. 114—115 °C (Found: C, 56.3; H, 4.65; N, 14.45. $C_{18}H_{18}N_4O_6$ requires C, 55.95; H, 4.7; N, 14.5%).

(b) *Benzyl* 2-*benzoyloxy*-2-*methyl*-3-*oxobutanoate* [cf. (21)]. Benzyl 2-methyl-3-oxobutanoate (2.0 g) in dry ether (5 cm^3) was stirred with sodium wire (220 mg) for 3 h. The resulting suspension was cooled to 0 °C and treated with a solution of benzoyl peroxide (2.35 g) in dry benzene (10 cm^3). The mixture was stirred for 2 h at 0 °C, treated with ether (25 cm^3), and washed with water (4 × 20 cm^3). The solution was dried ($MgSO_4$) and evaporated, and the residual oil was dissolved in methanol and kept at −10 °C for 24 h. The precipitated benzoyl peroxide was filtered off and the residue was distilled to give the ester (2.26 g, 71%) [as (21)], b.p. 143—145 °C at 0.03 mmHg. For analysis a *sample* was purified by bulb-tube distillation (0.02 mmHg; 200 °C) (Found: C, 70.1; H, 5.65. $C_{19}H_{18}O_5$ requires C, 69.9; H, 5.55%), $\nu_{max.}$ (neat) 1 730, 1 720, and 1 690 cm^{-1} (CO), δ 1.83 (3 H, s, *Me*COBz), 2.37 (3 H, s, MeCO), 5.19 (2 H, s, $C_6H_5CH_2$), 7.29 (5 H, s, $C_6H_6CH_2$), and 7.37—8.09 (5 H, m, C_6H_5CO).

Synthesis of Benzyl [1,3-$^{13}C_2$]-2-*Benzoyloxy*-2-*methyl*-3-*oxobutanoate* (21).—(a) *Benzyl* [1-^{13}C]*acetate*. A solution of [1-^{13}C]acetic acid (92.5 atom %, 500 mg, 8.33 mmol) in *NN*-dimethylacetamide was treated with tetramethylammonium hydroxide (20% solution in methanol, 5.2 g, 11.4 mmol) and a solution of benzyl bromide (5.7 g, 33.3 mmol) in methanol (2 cm^3). The heavy precipitate formed was dispersed using a vortex mixer. The mixture was allowed to stand for 1 h, toluene (20 cm^3) was added, and the mixture filtered. The precipitate was washed with toluene (20 cm^3) and the combined toluene solutions were washed with water (2 × 40 cm^3), dried ($MgSO_4$), and evaporated to give an oil (5.6 g). This was shown by g.l.c. [silicone grease (15%) on Chromosorb W (80—100 mesh), 5 ft × $\frac{1}{8}$ in, 200 °C, argon carrier gas, flow rate 30 cm^3 min^{-1}] to consist of a mixture of benzyl bromide, benzyl acetate, and benzyl methyl ether. The crude product (5.6 g) was applied to a column of silica gel (200 g; 60—120 mesh; 2.5 × 60 cm) and eluted with toluene–light petroleum (b.p. 40—60 °C) (1 : 1, 1.5 dm^3), toluene–chloroform (1 : 1, 1.5 dm^3), chloroform (1.5 dm^3), and methanol (1.5 dm^3). Fractions (10 cm^3) were collected and examined by g.l.c. Fractions 20—55 were combined to give benzyl bromide (2.65 g). Fractions 340—493 were combined to give a mixture of benzyl [1-^{13}C]acetate (820 mg) and benzyl methyl ether (210 mg). The mixture was used in the following step without further purification.

(b) *Benzyl* [1,3-$^{13}C_2$]-3-*oxobutanoate*. The mixture of

benzyl [1-^{13}C]acetate (800 mg, 5.3 mmol) and benzyl methyl ether (200 mg) was treated with finely powdered lithium hydride (63 mg, 7.9 mmol) and the mixture was heated under reflux at 130 °C for 6.5 h. After 2 h the orange mixture solidified. The cooled mixture was suspended in ether (25 cm^3) and the suspension was shaken with a mixture of acetic acid (470 mg, 7.86 mmol) and water (15 cm^3). The ethereal layer was washed with saturated sodium hydrogencarbonate solution (15 cm^3) and water (15 cm^3). The combined aqueous washings were re-extracted with chloroform (30 cm^3) and the combined organic solutions were dried ($MgSO_4$) and evaporated to give an oil (881 mg) from which pure benzyl [1,3-$^{13}C_2$]-3-oxobutanoate (229 mg, 46%) was isolated by preparative h.p.l.c. [Bondapak C_{18}–Porasil B, 8 ft × $\frac{3}{8}$ in, eluting solvent methanol–water (1 : 1)], δ 2.21 (3 H, d, *J* 6.3 Hz, Me^{13}CO), 3.46 (2 H, dd, *J* 6.6, 7.4 Hz, $^{13}COCH_2$ ^{13}CO), 5.14 (2 H, d, *J* 2.9 Hz, $^{13}CO{\cdot}O{\cdot}CH_2$), and 7.38 (5 H, s, C_6H_5) (the spectrum contained minor peaks due to [1-^{13}C] (6.9%), [3-^{13}C] (6.9%), and unlabelled (0.6%) ester).

(c) *Benzyl* [1,3-$^{13}C_2$]-2-*methyl*-3-*oxobutanoate* (20). Benzyl 3-oxobutanoate {[1,3-$^{13}C_2$] (85.6%), [1-^{13}C] (6.9%), [3-^{13}C] (6.9%), unlabelled (0.6%); 50 mg, 0.26 mmol} was diluted with unlabelled ester (250 mg, 1.3 mmol) and stirred with fine sodium wire (36 mg, 1.57 mmol) in dry ether (4 cm^3) for 2 h. Iodomethane (3 cm^3, 21 mmol) was added and the mixture boiled under reflux for 3.5 h. The cooled mixture was treated with ether (5 cm^3). The precipitated solid was filtered off and washed with dry ether (10 cm^3). The combined ethereal solutions were dried ($MgSO_4$) and evaporated to give benzyl [1,3-$^{13}C_2$]-2-methyl-3-oxobutanoate (249 mg, 78%). The product was shown (n.m.r.) to be of >93% purity and was used in the subsequent step without further purification.

(d) *Benzyl* [1,3-$^{13}C_2$]-2-*benzoyloxy*-2-*methyl*-3-*oxobutanoate* Benzyl 14.3% [1,3-$^{13}C_2$]-2-methyl-3-oxobutanoate (233 mg, 1.12 mmol) in dry ether (3 cm^3) was stirred with sodium wire (26 mg, 1.13 mmol) for 3 h. The resulting mixture was stirred for 3 h, washed with water (3 × 20 cm^3), dried ($MgSO_4$), and evaporated. The product, benzyl 14.3% [1,3-$^{13}C_2$]-2-benzoyloxy-2-methyl-3-oxobutanoate (157 mg, 43%) was isolated by h.p.l.c., as above.

Rearrangement of [1,3-$^{13}C_2$]-2-*Methyl*-3-*oxobutanoate in Alkaline Solution*.—A solution of benzyl 14.3% [1,3-$^{13}C_2$]-2-methyl-3-oxobutanoate (87 mg, 0.27 mmol) in methanol (0.45 cm^3) was stirred and treated dropwise with a solution of sodium hydroxide (0.27 cm^3, 2.0M) over 10 min. After a further 10 min the methanol was evaporated under reduced pressure and water (0.13 cm^3) was added to the residue which was filtered through a cotton wool plug into a 5-mm n.m.r. tube. A 25.2-MHz pulsed Fourier-transform ^{13}C n.m.r. spectrum was recorded with [$^2H_3$]methanol as an internal reference standard. Sodium hydroxide solution (0.05 cm^3, 2.0M) was added to the solution in the n.m.r. tube to bring the concentration of alkali to 0.2M. Further spectra were recorded after 15, 45, and 90 min.

Ethyl 2-*Benzoyloxy*-2-*methyl*-3-*oxobutanoate* (15).—This ester was prepared from ethyl 2-methyl-3-oxobutanoate and iodomethane by the method described above for the preparation of ester (23) and in similar yield. The product was purified by bulb-tube distillation at 150—200 °C and 0.05 mmHg (Found: C, 63.85; H, 6.1. $C_{14}H_{16}O_5$ requires C, 63.6; H, 6.1%), $\nu_{max.}$ (neat) 1 757 (CO·OEt), 1 737 (sh, ArCO), and 1 725 (MeCO) cm^{-1}, δ_H 1.2 (3 H, t, *J* 6 Hz, *Me*CH_2), 1.77 (3 H. s, *Me*COBz), 2.35 (3 H, s, *Me*CO), 4.14 (2 H, q, *J* 6 Hz, MeCH_2), and 7.2—8.0 (5 H, m, C_6H_5), δ_C 14.0 (C-7), 19.9 (C-5), 25.9 (C-4), 62.3 (C-6), 85.8 (C-2), 129.1, 129.4, 129.8, 133.6 (C-2′—7′), 164.9, 167.5 (C-1,-1′), and 201.2 p.p.m. (C-3).

We thank the S.R.C. for a CAPS studentship (to C. J. R. H.) and Roche Products Ltd. for financial support. The kinetic ^{13}C experiments were carried out with the kind assistance of Dr. C. H. Hassall and Dr. W. A. Thomas.

[8/1164 *Received*, 23*rd June*, 1978]

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